

Synthesis Based on Affinity Separation (SAS): Separation of Products Having Barbituric Acid Tag from Untagged Compounds by Using Hydrogen Bond Interaction

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Abstract: A new method is described for affinity purification of synthetic compounds based on molecular recognition between bis(2,6-diaminopyridine)amide of isophthalic acid and a barbituric acid derivative. The desired compounds possessing the barbituric acid derivative as a tag were readily isolated from the reaction mixture by the following procedure. After each reaction cycle, the reaction mixture was applied to the polystyrene column possessing bis(2,6-diaminopyridine)amide of isophthalic acid as an artificial receptor. The compound possessing the barbituric acid tag was selectively adsorbed on the column, whereas other impurities without the tag such as excess reagents and byproducts were washed off. Subsequent desorption with CH_2Cl_2 -MeOH (1:1) afforded the desired compound with high purity. This new strategy was applied to the synthesis of a heterocycle, peptides, and oligosaccharides.

Key words: affinity purification, molecular recognition, molecular tag, oligosaccharide synthesis, high throughput synthesis

Polymer supported synthesis has greatly simplified work-up and purification procedure of organic reactions to establish combinatorial and parallel synthesis.¹ In solid-phase synthesis, the synthetic product at each step on solid support is immediately separated from a reaction mixture by filtration. Although utility of solid-phase synthesis has been confirmed, there still exist several disadvantages: i) some of conventional reaction conditions in solution are not compatible with reactions on solid-supports, ii) the reaction rates on solid-supports are generally lower than those of the corresponding reactions in solutions. In liquid-phase synthesis using soluble polymer support, the reactivity of compounds on the support is similar to that in solution but additional step, i.e., precipitation of the polymer-supported products, is required for their isolation.^{1b}

Polymer-supported reagents and scavenger resins have made solution synthesis efficient but this strategy is not always effective for multistep synthesis because of the limited availability of the reagents.²

Recently, "tag" methodology has been developed as a hybrid method of solid-phase and solution-phase synthesis.³ In this methodology, a compound having a tag group is easily separated from untagged molecules by using the affinity of the tag with a respective specific functionality. Several tags such as fluororous affinity tags,^{3a-c} hydrophobic tags,^{3d-f} a 2-pyridylsilyl tag for acidic extraction,^{3g} and an anthracene tag for Diels-Alder resin capture/release^{3h} have been reported. The "tag" methodology has advantages over polymer-supported synthesis in the following points. Namely, monitoring of reaction is easy by simple and routine methods such as TLC and HPLC. Additional purification of tagged intermediates is possible by other methods after any reaction steps.

We previously reported a new "tag" strategy, termed "synthesis based on affinity separation (SAS)", in which the desired tagged compound was separated from the reaction mixture by solid-phase extraction using specific molecular recognition (Figure 1).⁴ In that work, we employed the interaction between a crown ether (32-crown-10) and ammonium ion for SAS.

In the present study, we investigated a new SAS method by using interaction via multiple hydrogen bonds between a guest residue and its artificial receptor. From many artificial receptors reported, we employed Hamilton's receptor, i.e., bis(2,6-diaminopyridine)amide of isophthalic acid, which forms tight complex with barbituric acid via

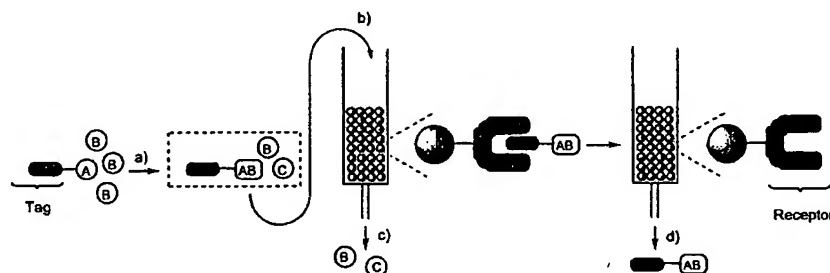


Figure 1 Synthesis based on affinity separation. A: substrate, B: reagent, AB: product, C: byproduct. a) reaction in solution, b) adsorption of tagged product, c) elution of excess reagent and untagged byproduct, d) desorption of tagged product.

six hydrogen bonds.⁵ A barbituric acid moiety was used as a tag and the receptor moiety was bound to polystyrene support (Figure 2).

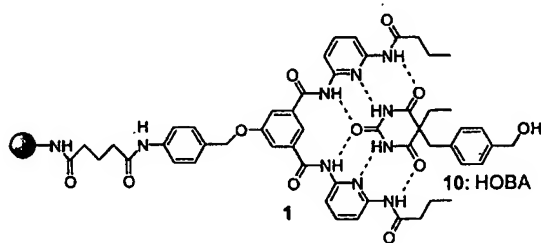
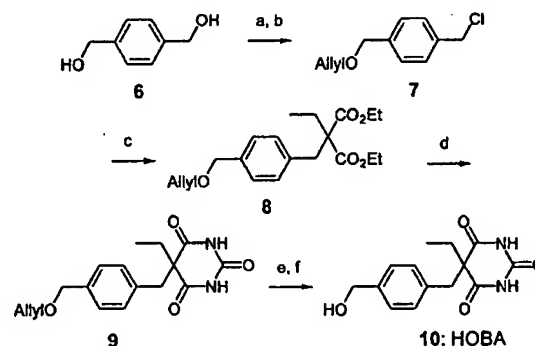


Figure 2 Host-guest interaction of a polymer-supported receptor with the barbituric acid tag.

Polymer-supported artificial receptor (1) was synthesized from 5-hydroxyisophthalic acid (2) as illustrated in Scheme 1.⁶ A highly cross-linked macroporous polystyrene resin ArgoPore™-NH₂ (1.16 mmol/g) was used as a support since it gave better results than the usual 1% cross-linked gel-type polystyrene resin in the previous study. The barbituric acid tag 10 (HOBA) was synthesized as shown in Scheme 2.⁷

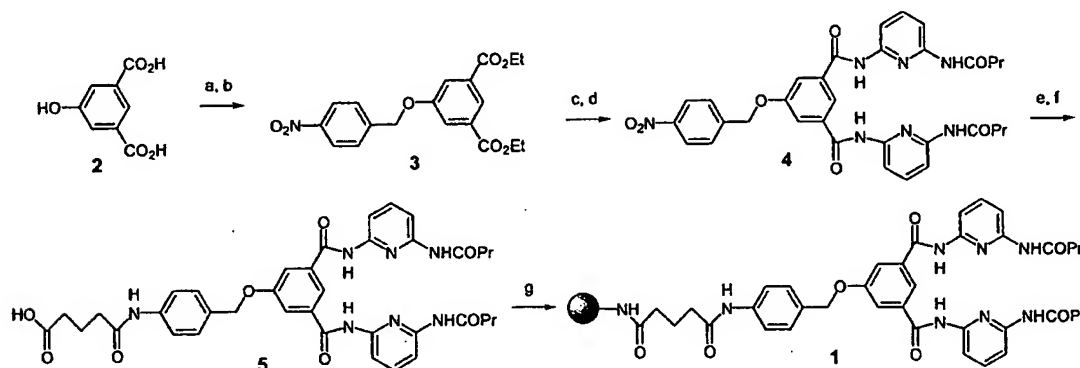
The tagged compounds were effectively retained in the resin column 1 by using nonpolar eluents such as CH₂Cl₂, CHCl₃, and toluene, which favors the formation of a complex through hydrogen bonds. Since polar solvents disfavor the complex formation, the tagged compounds were readily desorbed from the column by using CH₂Cl₂-methanol as an eluent. The separation scale was 0.1 mmol–0.2 mmol for peptides and heterocycle and 0.05 mmol–0.11 mmol for oligosaccharides with 7 g of the resin 1.

This methodology was first applied to the synthesis of small peptides (Scheme 3).⁸ The tag moiety 10 was coupled to the starting Fmoc amino acid 11 by using diisopro-



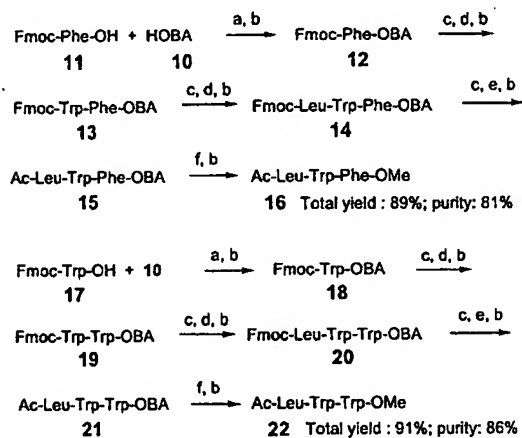
Scheme 2 Reagents and conditions: a) xylylene glycol (2.0 equiv.), NaH (2.2 equiv.), allylBr (1.0 equiv.), THF, 60°C, 7 h, 63%; b) [Me₂NCl]⁺Cl⁻ (1.3 equiv.), MeCN, reflux, 30 min, 99%; c) diethyl ethylmalonate (1.06 equiv.), KOBu^t (1.06 equiv.), toluene, reflux, 6.5 h, 98%; d) urea (10 equiv.), NaH (4 equiv.), DMSO, rt, 14 h, 81%; e) [Ir(COD)(PMePh₃)]⁺PF₆⁻ (0.045 equiv.), H₂, THF, rt, 100 min, f) I₂/H₂O, rt, 30 min, 87%.

pylcarbodiimide (DIC) and a catalytic amount of 4-(dimethylamino)pyridine (DMAP) in CH₂Cl₂. The reaction mixture was directly applied to the resin column 1. The tagged 12 was retained in the column, whereas all untagged compounds such as excess 11, DIC, DMAP, and *N,N*-diisopropylurea were washed out from the column simply by elution with CH₂Cl₂. Elution of 12 with CH₂Cl₂-methanol (1:1) and concentration gave purified 12. Fmoc group in 12 was then removed with 5% piperidine in DMF. Peptide condensation was carried out by the DIC method in CH₂Cl₂ to give dipeptide 13, which was purified by the same affinity separation. After tripeptide 14 was formed in a similar manner, the removal of the Fmoc group, *N*-acetylation, and purification by the affinity separation afforded 15. The tag moiety of 15 was then removed by transesterification to give 16, which was separated from the barbituric acid tag 10 by the affinity separation: the desired compound 16 passed through the



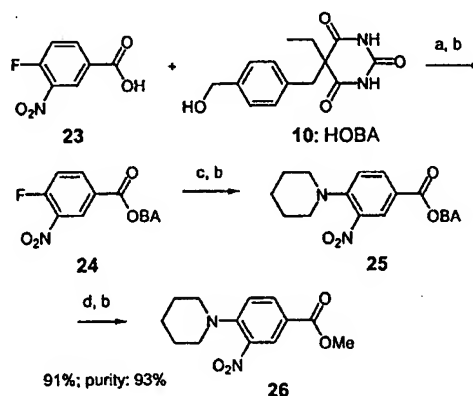
Scheme 1 Reagents and conditions: a) AcCl, EtOH, reflux, 4 h, 98%; b) *p*-nitrobenzyl bromide (1.12 equiv.), K₂CO₃, acetone, reflux, 2 h, 97%; c) *n*-BuLi (1.95 equiv.), 2,6-diaminopyridine (2.0 equiv.), THF, -78 °C to -60 °C, 8 h, 91%; d) PrCOCl (1.4 equiv.), DIEA, THF, rt, 4 h, 92%; e) NiCl₂·6H₂O, NaBH₄, MeOH, THF, rt, 1.5 h; f) glutaric anhydride (2.0 equiv.), THF, rt, 5 h, 60% over two steps; g) ArgoPore™-NH₂ (1.16 mmol/g), DIC, DMAP, DMF, rt, 3 d.

column whereas **10** was adsorbed to the column. The overall yield of the tripeptide **16** was 89% (from HOBA (**10**)) with 81% purity (judged by HPLC analysis at 220 nm). Tripeptide Ac-Leu-Trp-Trp-OMe (**22**) (91% yield with 86% purity) was also synthesized by the same SAS strategy.



Scheme 3 Reagents and conditions: a) Fmoc-amino acids (1.25 equiv.), DIC (2 equiv.), DMAP, CH_2Cl_2 ; b) affinity separation; c) 5% piperidine in DMF, rt, 8 min; d) Fmoc-amino acids (1.25 equiv.), DIC (2 equiv.), CH_2Cl_2 ; e) Ac_2O , TEA, CH_2Cl_2 ; f) 0.2 M NaOMe in MeOH, rt, 10 min.

This new synthetic protocol was then applied to synthesis of *N*-arylpiperidine (Scheme 4). Since 1-methyl-2-pyrrolidone (NMP), which reduces the interaction of the artificial receptor with the tag, was used as a solvent for the aromatic nucleophilic substitution of **24** with piperidine,⁹ the reaction mixture was first diluted with CH_2Cl_2 and then subjected to the affinity separation. Purification of

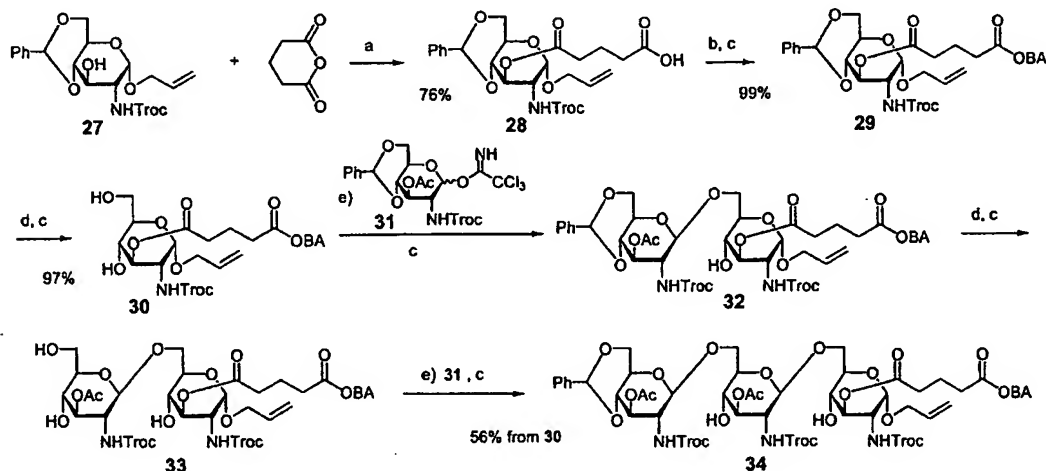


Scheme 4 Reagents and conditions: a) DIC, DMAP, CH_2Cl_2 ; b) affinity separation; c) piperidine (1.5 equiv.), NMP, rt, 2h; d) 0.2 M MeONa in MeOH, rt, 10 min.

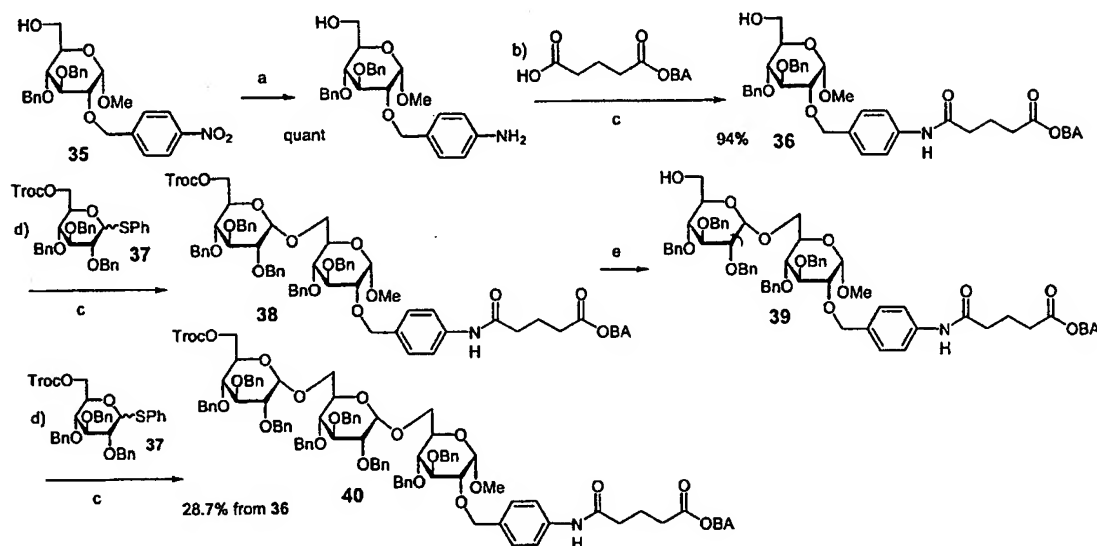
arylpiperidine **25** was thus effected without additional steps for the removal of NMP. Transesterification and isolation by affinity separation gave **26** in overall 91% yield.¹⁰

We then investigated oligosaccharide synthesis by the present SAS strategy (Scheme 5 and 6). The keys for the success were the following two points: i) the tag moiety was stable under glycosylation conditions and ii) oligosaccharides possessing many protective groups were effectively adsorbed on the resin.

The first example is synthesis of β -(1-6)oligoglucosamine (Scheme 5). The tag **10** was attached to the starting *N*-trichloroethoxycarbonyl (Troc) glucosamine **27** through a glutaric acid spacer. Removal of the benzylidene group of **29** gave a glycosyl acceptor **30**. β -Selective glycosylation of **30** with glycosyl trichloroacetimidate **31** (1.5 equiv. to **30**) was effected by using TMSOTf as a catalyst by virtue



Scheme 5 Reagents and conditions: a) pyridine, DMAP, CH_2Cl_2 , 3.5 h; b) HOBA (**10**), DIC, DMAP, CH_2Cl_2 , 1.5 h; c) affinity separation; d) 5% TFA, 1% H_2O in CH_2Cl_2 , 0 °C; e) TMSOTf (0.1 equiv.), MS4A, CH_2Cl_2 , -20 °C, 10 min.



Scheme 6 Reagents and conditions. a) $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, NaBH_4 , MeOH ; b) DIC , CH_2Cl_2 ; c) affinity separation; d) PhIO , TMSOTf , $\text{Et}_2\text{O}/\text{dioxane}$; e) Zn-Cu couple, AcOH .

of the neighboring participation of the *N*-Troc group to afford the disaccharide 32.¹¹ Removal of the benzylidene from 32 followed by glycosylation with 31 (5 equiv.) gave trisaccharide 34. After each reaction step, the product was rapidly separated from the reaction mixture by the affinity separation in this synthesis, too. Since there are two and three hydroxy groups in acceptors 30 and 33, respectively, some undesired byproducts were formed by their glycosylation at more hindered 4-positions. These byproducts were not separated by affinity separation like solid-phase synthesis, since they had the tag moiety. The desired trisaccharide 34 was, however, readily purified by additional simple silica-gel chromatography. The purified 34 was thus obtained in 56% yield from 30 after silica-gel column chromatography.¹² In the case of solid-phase synthesis, purification is only possible after products are cleaved from solid-supports. By using the present SAS method, products can be purified by affinity separation as well as other purification methods. Purified synthetic intermediates can be subjected to next reaction. This is important feature of the present methodology.

As shown in Scheme 6, oligosaccharide synthesis was carried out under the conditions for α -selective glycosylation by virtue of the solvent effect of ether using 2-*O*-benzylated thioglycosyl donors. Since the solubilities of glycosyl acceptors having the barbituric acid tag in ether were low, a mixture of ether-dioxane was used as the reaction solvent.¹³ We previously found combinations of iodosobenzene (PhIO) and various acids effectively promote glycosylation using thioglycosyl donors.¹⁴ Among those found to be satisfactory, the combination of PhIO and TMSOTf was employed in the present study.¹⁵

The tagged acceptor 36 was prepared from 4-nitrobenzyl glucoside 35 via reduction of the nitro group followed by

coupling with the tag moiety (Scheme 6).¹⁶ Glycosylation of the tagged acceptor 36 with excess phenyl 6-*O*-Troc-thioglycoside 37 (1.5 equiv.) using PhIO and TMSOTf gave disaccharide 38 ($\alpha:\beta = 10:1$).¹⁷ The 6-*O*-Troc group of 38 was removed and the resulting 6-*O*-free disaccharide 39 was then glycosylated with 37 to give the trisaccharide 40 (28.7% from 36).¹⁸ After each reaction step, the product was rapidly separated from the reaction mixture by the affinity separation. Since the acylaminobenzyl linker was partly cleaved at the glycosylation step, the final product 40 was purified by silica-gel column chromatography after the affinity separation.

In summary, we have developed a new synthetic strategy based on affinity separation. The present method can be applied to the synthesis of relatively large molecules such as protected oligosaccharides owing to the strong affinity of the barbituric acid tag with its artificial receptor, whereas previous tag strategies have been applied to synthesis of small molecules.¹⁹ The strategy greatly facilitates the purification procedures in solution-phase synthesis and is expected to be particularly useful for multiple parallel synthesis and combinatorial library preparation where application of solid-phase synthesis is difficult.

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References and Notes

- (1) a) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* 1996, 96, 555. b) Gravert, D. J.; Janda, K. D.; *Chem. Rev.* 1997, 97, 489. c) Dolle, R. E.; Nelson, Jr. K. H. *J. Comb. Chem.* 1999, 1, 235. d) Dolle, R. E. *J. Comb. Chem.* 2000, 2, 383.
- (2) a) Kaldor, S. W.; Siegel, M. G. *Curr. Opin. Chem. Biol.* 1997, 1, 101. b) Booth, J.; Hodges, J. C. *Acc. Chem. Res.* 1999, 32, 18. c) Thompson, L. A. *Curr. Opin. Chem. Biol.* 2000, 4, 324.
- (3) a) Curran, D. P. *Angew. Chem., Int. Ed.* 1998, 37, 1174. b) Curran, D. P.; Luo, Z. *J. Am. Chem. Soc.* 1999, 121, 9069. c) Horváth, I. T. *Acc. Chem. Res.* 1998, 31, 641. d) Ramage, R.; Swenson, H. R.; Shaw, K. T. *Tetrahedron Lett.* 1998, 39, 8715. e) Hay, A. M.; Hobbs-Dewitt, S.; MacDonald, A. A.; Ramage, R. *Tetrahedron Lett.* 1998, 39, 8721. f) Nilsson, U. J.; Fournier, E. J.-L.; Hindsgaul, O. *Bioorg. Med. Chem.* 1998, 6, 1563. g) Yoshida, J.; Itami, K.; Mitsudo, K.; Suga, S. *Tetrahedron Lett.* 1999, 40, 3403. h) Wang, X.; Parlow, J. J.; Porco Jr., J. A. *Org. Lett.* 2000, 2, 3509.
- (4) a) Zhang, S.-Q.; Fukase, K.; Kusumoto, S. *Tetrahedron Lett.* 1999, 40, 7479. b) Zhang, S.-Q.; Fukase, K.; Kusumoto, S. *Peptide Science 1999* (Ed: N. Fujii), the Japanese Peptide Society 2000, pp. 151-154.
- (5) a) Chang, S. K.; Hamilton, A. D. *J. Am. Chem. Soc.* 1988, 110, 1318. b) Moteshare, K.; Myles, D. C. *J. Am. Chem. Soc.* 1998, 120, 7328.
- (6) The experimental procedure for the preparation of the polymer supported receptor 1. Diethyl 5-(4-Nitrophenylmethoxy)isophthalate (3). The mixture of diethyl 5-hydroxyisophthalate (7.68 g, 32.2 mmol), *p*-nitrobenzyl bromide (7.80 g, 36.0 mmol), and K_2CO_3 (8.0 g, 58 mmol) in acetone (100 ml) was refluxed for 2 h. After the mixture was cooled to room temperature, insoluble materials were removed by filtration and the filtrate was concentrated in vacuo. The crystalline residue was recrystallized from ethyl acetate and hexane to give 3 (11.6 g, 96.5%). ESI-MS (positive) m/z 396.1 [(M+Na)⁺]. *N,N'*-Bis[2-(6-aminopyridyl)]-5-(4-nitrophenylmethoxy)isophthaldiamide. To a solution of 2,6-diaminopyridine (8.00 g, 73.3 mmol) in anhydrous THF (150 ml) was added 25.0 ml of *n*-butyl lithium in hexane (2.52 mol·l⁻¹) at -78 °C under N_2 . After the mixture was stirred for 20 min, a solution of 3 (6.00 g, 16.1 mmol) in anhydrous THF (55 ml) was added dropwise. The mixture was stirred at -78 °C to -60 °C for 6 h and water (30 ml) was added to quench the reaction. After addition of EtOAc (200 ml), the organic layer was washed with water and brine, dried over Na_2SO_4 . Parts of the desired product were crystallized from extracts. Filtration afforded 6.40 g of the product. The filtrate was concentrated in vacuo and the residue was purified by silica-gel column chromatography (EtOAc/hexane = 1:1) to give another 0.90 g of the product. Yield: 7.30 g (90.9%). ESI-MS (positive) m/z 500.2 [(M+H)⁺]. *N,N'*-Bis[2-(6-butylaminopyridyl)]-5-(4-nitrophenylmethoxy)isophthaldiamide (4). To a mixture of *N,N'*-bis[2-(6-aminopyridyl)]-5-(4-nitrophenylmethoxy)isophthaldiamide (3.32 g, 3.35 mmol) and *N,N*-diisopropylethylamine (2.5 ml, 14.4 mmol) in THF (80 ml) was added butanoyl chloride (2.0 ml, 19.3 mmol). The mixture was stirred at room temperature for 4 h under N_2 . After addition of water (50 ml) and EtOAc (200 ml), the organic layer was washed with 0.5 M NaOH (50 ml × 1) and brine (100 ml × 2), dried over Na_2SO_4 , and concentrated. The residue was purified by silica-gel column chromatography (CHCl₃/acetone = 10:1) to give 4 (3.8 g, 91.8%). ESI-MS (positive) m/z 662.3 [(M+Na)⁺]. ¹H NMR (600 MHz, DMSO), δ = 10.50 (2H, s, 2 × CONH), 10.09 (2H, s, 2 × CONH), 8.30 (2H, d, J = 8.5 Hz, PhNO₂), 8.16 (1H, s, Ph), 7.83-7.75 (10H, m, Ar-H), 5.47 (2H, s, -OCH₂-), 2.37 (4H, t, J = 7.3 Hz, 2 × -COCH₂-), 1.59 (4H, m, 2 × CH₂), 0.90 (6H, t, J = 7.3 Hz, 2 × CH₃). *N,N'*-Bis[2-(6-butylaminopyridyl)]-5-[4-(4-carboxylbutyryl)phenylmethoxy]isophthaldiamide (5). To a solution of NiCl₂·6H₂O (0.3 g, 1.26 mmol) in MeOH (80 ml) was added of NaBH₄ (0.1 g, 2.64 mmol) (the color of solution changed from green to black) and a solution of 4 (2.0 g, 3.13 mmol) in THF (40 ml) was added. NaBH₄ (4.0 g, 106 mmol) was then added to the mixture by portions for 1.5 h. After that 1M NaOH (20 ml) and EtOAc (80 ml) were added, the organic layer was washed with brine (60 ml × 3), dried over Na_2SO_4 , and concentrated in vacuo. To the solution of the residue in THF (30 ml) was added glutaric anhydride (1.0 g, 8.77 mmol) and the mixture was stirred at room temperature for 5 h. The desired 5 precipitated was collected by filtration and washed with THF to give 1.10 g of 5. The filtrate was concentrated and the residue was purified by silica-gel column chromatography (CHCl₃/MeOH = 20:1) to give another 0.25 g of the product. Yield 1.35 g (59.7%). ESI-MS m/z 722.3 (negative) [(M-H)⁻]. ¹H NMR (600 MHz, DMSO), δ = 10.49 (2H, s, 2 × CONH), 10.11 (2H, s, 2 × CONH), 10.00 (1H, s, CONH), 8.13 (1H, s, Ph), 7.86-7.72 (5H, Ar-H), 7.62 (2H, d, J = 7.7 Hz, PhNHCO), 7.42 (2H, d, J = 7.7 Hz, PhNHCO), 5.21 (2H, s, -OCH₂-), 2.37 (6H, m, 3 × CH₂CO), 2.26 (2H, t, CH₂CO), 1.75 (4H, m, 2 × CH₂), 1.61 (4H, m, 2 × CH₂), 0.90 (6H, t, J = 6.9 Hz, 2 × CH₃). Preparation of the polymer supported receptor 1. To a suspension ArgoPore-NH₂-TM (1.16 mmol/g) (1.00 g, 1.16 mmol of NH₂ group), 5 (1.25 g, 1.73 mmol), and DMAP (5.0 mg, 0.04 mmol) in DMF (10 ml) was added DIC (545 μ l, 3.48 mmol) at room temperature. After 3 d of agitation, the resin was washed with DMF (20 ml × 3), CH₂Cl₂-MeOH 1:1 (20 ml × 3), and CH₂Cl₂ (20 ml × 3) and dried under reduced pressure to give the receptor-bound resin (1.42 g, 50.2%).
- (7) The experimental procedure for the preparation of the tag 10. 4-Allyloxymethylbenzyl Alcohol. To a solution of *p*-xylylene glycol (6) (11.1 g, 80.3 mmol) in anhydrous THF (200 ml) was added NaH (60% oil suspension, 3.52 g, 88.0 mmol). The mixture was stirred at room temperature for 1 h. Allyl bromide (3.46 ml, 40.0 mmol) was added to the solution and the mixture was stirred at 60 °C for 7 h. After addition of EtOAc and aqueous saturated citric acid solution, the organic layer was washed with water and brine, dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by silica-gel column chromatography (CHCl₃/acetone = 1:0 to 9:1) to give 5.64 g (63.4%) of the desired product. ESI-MS (positive) m/z 178.44 (M⁺); 196.44 [(M+H)⁺]. 4-Allyloxymethylbenzyl Chloride (7). To a solution of chloromethylenedimethylammonium chloride (1.30 g, 10.2 mmol) in anhydrous CH₃CN (10 ml) was added dropwise a solution of 4-allyloxymethylmethanol (1.30 g, 7.29 mmol) in anhydrous CH₃CN (10 ml) under N_2 and the mixture was refluxed for 40 min. After the mixture was cooled to room temperature, ice-cold water (10 ml) was added. The mixture was extracted with EtOAc and the organic layer was washed with water, dried over K_2CO_3 , and concentrated in vacuo to give 7 (1.14 g, 98.6%). Diethyl 2-(4-Allyloxymethylbenzyl)-2-ethylmalonate (8). To a solution of diethyl ethylmalonate (2.30 g, 12.2 mmol) in anhydrous toluene (40 ml) was added potassium *t*-butoxide (1.41 g, 12.6 mmol) under N_2 . After the mixture was refluxed for 30 min, the solution of 7 (1.40 g, 7.12 mmol) in toluene (20 ml) was added. The mixture was refluxed for 6.5 h and then cooled to room temperature. After addition of water and EtOAc, the organic layer was washed with 0.5 M HCl, saturated NaHCO₃ solution, and brine, dried over Na_2SO_4 , and

concentrated in vacuo. The residue was purified by silica-gel column chromatography (hexane/EtOAc = 20:1 to 10:1) to afford **8** (3.90 g, 97.7%). ESI-MS (positive) m/z 349.35 [(M+H)⁺, 18%]; 366.35 [(M+H₂O)⁺, 40%]; 371.30 [(M+Na)⁺, 100%]. ¹H NMR (500 MHz, CDCl₃), δ = 7.22 (2H, d, J = 7.7 Hz, Ar-H), 7.07 (2H, d, J = 8.0 Hz, Ar-H), 5.94 (1H, m, CH₂=CH-CH₂-), 5.29 (1H, m, CH₂=CH-CH₂-), 5.19 (1H, m, CH₂=CH-CH₂-), 4.47 (2H, s, -OCH₂PhCH₂-), 4.18 (4H, m, 2 \times -OCH₂CH₂-), 4.01 (2H, m, CH₂=CH-CH₂-), 3.23 (2H, s, -OCH₂PhCH₂-), 1.82 (2H, q, J = 7.6 Hz, -CH₂CH₃), 1.24 (6H, t, J = 7.1 Hz, 2 \times -OCH₂CH₂-), 0.92 (3H, t, J = 7.6 Hz, -CH₂CH₃).

5-Ethyl-5-(4-allyloxymethylbenzyl)barbituric Acid (9). To a solution of urea (7.00 g, 117 mmol) in DMSO (25 ml) was added NaH (60% oil suspension, 2.0 g, 50 mmol) under N₂. After the mixture was stirred at room temperature for 30 min, **8** (4.10 g, 11.8 mmol) was added. The mixture was stirred at room temperature for 14 h and then poured into 20 g of ice. The mixture was acidified with 2M HCl and then extracted with EtOAc. The organic layer was washed with water, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica-gel column chromatography (hexane/EtOAc = 3:1 to 2:1) to give **9** (3.00 g, 80.6%). ESI-MS (positive) m/z 339.15 [(M+Na)⁺]. ¹H NMR (600 MHz, CDCl₃), δ = 7.86 (2H, s, NH), 7.22 (2H, d, J = 7.7 Hz, Ar-H), 7.08 (2H, d, J = 8 Hz, Ar-H), 5.93 (1H, m, CH₂=CH-CH₂-), 5.29 (1H, m, CH₂=CH-CH₂-), 5.20 (1H, m, CH₂=CH-CH₂-), 4.46 (2H, s, -OCH₂PhCH₂-), 4.00 (2H, m, CH₂=CH-CH₂-), 3.27 (2H, s, -OCH₂PhCH₂-), 2.20 (2H, q, J = 7.6 Hz, -CH₂CH₃), 0.92 (3H, t, J = 7.6 Hz, -CH₂CH₃).

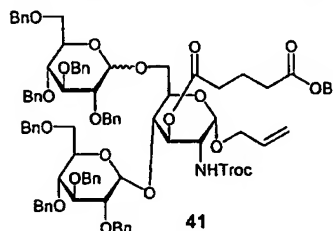
5-Ethyl-5-(4-hydroxymethylbenzyl)barbituric Acid (HOBA) (10). To a degassed solution of **9** (2.89 g, 9.13 mmol) in anhydrous THF (100 ml) was added [Ir(COD)(PMePPh₂)₂]⁺PF₆⁻ (350 mg, 0.045 equiv) and the solution was stirred under H₂ at room temperature for 5 min (the color of the solution changed from red to yellow) and then for 100 min under N₂. Water (45 ml) and iodine (4.4 g) was added to the mixture successively. After the mixture was stirred for 30 min, aqueous 10% Na₂S₂O₃ solution (10 ml) was added to quench the reaction. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by silica-gel column chromatography (CHCl₃/MeOH = 20:1) to give **10** (2.20 g, 87.3%). ESI-MS (positive) m/z 575.3 [(2M+Na)⁺]; 315.1 [(M+K)⁺]; 299.2 [(M+Na)⁺]. ¹H NMR (600 MHz, DMSO), δ = 7.18 (2H, d, J = 8 Hz, Ar-H), 6.94 (2H, d, J = 8 Hz, Ar-H), 5.15 (t, 1H, OH), 4.24 (2H, m, -OCH₂PhCH₂-), 3.07 (2H, s, -OCH₂PhCH₂-), 1.97 (2H, q, J = 7.6 Hz, -CH₂CH₃), 0.76 (3H, t, J = 7.6 Hz, -CH₂CH₃).

- (8) General procedure for affinity separation. After completion of the reaction, the reaction mixture was directly applied to the resin 1 column (7.0 g: 1.5 cm \times 7 cm, CH₂Cl₂) unless otherwise noted. After untagged compounds were washed off with CH₂Cl₂ (70 ml), the tagged compound was eluted with MeOH-CH₂Cl₂ (1:1) (30 ml). Evaporation of the solvents afforded the desired product having the tag.
- (9) Dankwardt, S. M.; Newman, S. R.; Krstenansky, J. L. *Tetrahedron Lett.* 1995, 36, 4923.
- (10) The experimental procedure for the preparation of methyl 4-piperidino-3-nitrobenzoate (**26**). To a solution of HOBA (**10**) (27.6 mg, 0.100 mmol), 4-fluoro-3-nitrobenzoic acid (22.0 mg, 0.119 mmol), and DMAP (1 mg, 8 μ mol) in anhydrous CH₂Cl₂ (3 ml) was added DIC (30 μ l, 0.19 mmol). The mixture was stirred at room temperature for 2 h and then applied to the affinity separation to give 5-ethyl-5-[4-(4-fluoro-3-nitrobenzoyl)oxymethylbenzyl]barbituric acid (**24**). To a solution of **24** in *N*-methylpyrrolidone (NMP) (0.8 ml)

was added piperidine (20 μ l, 0.20 mmol). The mixture was stirred at room temperature for 2 h, diluted with CH₂Cl₂ (8 ml), and then applied to the affinity separation. The desired 5-ethyl-5-[4-(4-piperidino-3-nitrobenzoyl)oxymethylbenzyl]barbituric acid (**25**) was eluted at both CH₂Cl₂ and CH₂Cl₂-MeOH (1:1) fractions. The CH₂Cl₂ fraction containing **25** was concentrated and then applied to the affinity separation again. The CH₂Cl₂-MeOH (1:1) fractions were combined and concentrated. Compound **25** thus obtained was dissolved in 0.2 M MeONa in MeOH (2.0 ml) and the mixture was stirred at room temperature for 30 min. After addition of AcOH (3 drops), the mixture was concentrated in vacuo. The residue was dissolved in CHCl₃ and then applied to the affinity separation. The desired **26** was eluted with CHCl₃, whereas HOBA (**10**) was eluted with CH₂Cl₂/MeOH (1:1). Evaporation of the solvent afforded **26** (24.0 mg, 90.9%). ESI-MS (positive) m/z 265.2 [(M+H)⁺]. ¹H NMR (600 MHz, CDCl₃), δ = 8.42 (1H, s, Ar-H), 8.02 (1H, d, J = 8.8 Hz, Ar-H), 7.05 (1H, d, J = 8.8 Hz, Ar-H), 3.90 (3H, s, CH₃), 3.15 (4H, t, NCH₂), 1.72 (4H, m, CH₂), 1.51 (2H, m, CH₂).

- (11) Liu, W.-C.; Oikawa, M.; Fukase, K.; Suda, Y.; Kusumoto, S. *Bull. Chem. Soc., Jpn.* 1999, 72, 1377.
- (12) The experimental procedure for the preparation of trisaccharide **34**. The mixture of the glycosyl acceptor **30** (69.2 mg, 0.09 mmol), the glycosyl donor **31** (85 mg, 0.135 mmol), and MS 4A in CH₂Cl₂ (4 ml) was stirred at room temperature for 1 h under N₂ atmosphere and then cooled to -20 °C. TMSOTf (4.0 μ l, 22 μ mol) was added and the mixture was stirred at the same temperature for 10 min. The mixture was directly applied to the affinity separation to give disaccharide **32**. Disaccharide **32** was dissolved in CH₂Cl₂ (4 ml) containing 5% TFA and 1% water. The mixture was stirred at 0 °C for 30 min, diluted with EtOAc (15 ml), washed with saturated NaHCO₃ solution and brine, dried over Na₂SO₄, and concentrated. The residue was applied to the affinity separation to afford disaccharide acceptor **33**. The mixture of **33** (59.2 mg, 0.0517 mmol), **31** (161 mg, 0.256 mmol), and MS 4A in CH₂Cl₂ (5 ml) was stirred at room temperature for 1 h under N₂, and then cooled to -20 °C. TMSOTf (4.0 μ l, 22 μ mol) was added and the mixture was stirred at the same temperature for 10 min. The mixture was directly applied to the affinity separation to give 80.1 mg of the crude product, which was then purified by silica-gel column chromatography to afford the pure trisaccharide **34** (47.0 mg, 56.0%). ESI-MS m/z 1630.0 [(M+Na)⁺]. ¹H NMR (500 MHz, CDCl₃), δ = 8.45 (2H, NH: barbituric acid), 7.45 (2H, m, Ph-H), 7.34 (3H, m, Ph-H), 7.20 (2H, d, J = 7.6 Hz, Ph-H), 7.09 (2H, d, J = 8.1 Hz, Ph-H), 6.28 (1H, br s, 2-NH: GlcN_C), 5.92 (1H, m, CH₂=CH-CH₂-), 5.51 (1H, s, PhCH=), 5.43 (1H, d, J = 9.9 Hz, 2-NH: GlcN_A), 5.37 (1H, br s, 2-NH: GlcN_B), 5.33 (1H, m, CH₂=CH-CH₂-), 5.24 (1H, m, CH₂=CH-CH₂-), 5.18 (1H, t, J = 9.7 Hz, H-3: GlcN_A), 5.13 (1H, t, J = 9.9 Hz, H-3: GlcN_C), 5.05 (1H, H-3: GlcN_B), 5.02 (1H, d, J = 14 Hz, -OCH₂PhCH₂-), 4.97 (1H, d, J = 14 Hz, -OCH₂PhCH₂-), 4.90 (d, J = 3.7 Hz, 1H, H-1: GlcN_A), 4.79-4.65 (6H, CCl₃CH₂- \times 3), 4.65 (1H, d, J = 8.3 Hz, H-1: GlcN_C), 4.63 (1H, d, J = 8.6 Hz, H-1: GlcN_B), 4.37 (1H, dd, J = 5.6 Hz, 10.6 Hz, H-6: GlcN_C), 4.22 (1H, m, CH₂=CH-CH₂-), 4.21 (1H, H-6: GlcN_B), 4.17 (1H, H-6: GlcN_A), 4.02 (1H, m, CH₂=CH-CH₂-), 3.96 (1H, H-5: GlcN_A), 3.95 (1H, H-2: GlcN_A), 3.84 (1H, H-6': GlcN_A), 3.82 (1H, H-6': GlcN_C), 3.80 (1H, H-2: GlcN_C), 3.79 (1H, H-6': GlcN_B), 3.71 (1H, H-4: GlcN_C), 3.70 (1H, H-4: GlcN_A), 3.62 (1H, H-2: GlcN_B), 3.53 (1H, H-5: GlcN_B), 3.52 (1H, H-5: GlcN_C), 3.51 (1H, H-4: GlcN_B), 3.26 (2H, m, -OCH₂PhCH₂-), 2.35-2.22 (4H, m, -COCH₂CH₂CH₂CO-), 2.18 (2H, q, J = 6.2 Hz, CH₂CH₂-), 2.10 (3H, s, CH₃CO-), 1.90 (2H, m, -

- COCH₂CH₂CH₂CO-), (3H, t, $J = 6.2$ Hz, CH₃CH₂-) (the saccharide residues are designated as GlcN_A, GlcN_B, and GlcN_C (glucosamine residue) from reducing end).
- (13) α -Promoting solvent effect of dioxane was reported. a) Demchenko, A. V.; Rousson, E.; Boons, G.-J. *Tetrahedron Lett.* 1999, 40, 6523. b) Rademann, J.; Schmidt, R. R. *J. Org. Chem.* 1997, 62, 3650.
- (14) a) Fukase, K.; Kinoshita, I.; Kanoh, T.; Nakai, Y.; Hasuoka, A.; Kusumoto, S. *Tetrahedron* 1996, 52, 3897. b) Fukase, K.; Nakai, Y.; Kanoh, T.; Kusumoto, S. *Synlett* 1998, 84.
- (15) α -Selectivity is expected to be increased by using combinations of PhIO with SnCl₂-AgClO₄, SnCl₄-AgClO₄, BiCl₃-AgClO₄, and SbCl₅-AgClO₄, though these reagents require careful handling.¹⁴
- (16) An acylaminobenzyl linker, which can be cleaved by DDQ oxidation, was used for solid-phase oligosaccharide synthesis. Fukase, K.; Nakai, Y.; Egusa, K.; Porco Jr., J. A.; Kusumoto, S. *Synlett* 1999, 1074.
- (17) We previously reported α -orienting effect of the 6-*O*-Troc group.¹⁴ Fukase, K.; Yoshimura, T.; Kotani, S.; Kusumoto, S. *Bull. Chem. Soc. Jpn.* 1994, 67, 473.
- (18) The experimental procedure for the preparation of trisaccharide 40. The mixture of the glycosyl acceptor 36 (97.4 mg, 0.114 mmol), phenyl thioglucoside 37 (122.8 mg, 0.171 mmol), PhIO (48 mg, 0.22 mmol), and MS 4A in diethyl ether (5.0 ml) and dioxane (4.0 ml) was stirred for 1 h at r.t. under N₂ then cooled to -10 °C. TMSOTf (20 μ l, 0.110 mmol) was added and the reaction mixture was stirred for 1 h. The reaction was quenched by addition of aqueous saturated NaHCO₃ solution and ethyl acetate. The organic layer was washed with brine, dried over Na₂SO₄, and concentrated. The residue was dissolved in CH₂Cl₂ and then subjected to the affinity separation. Since the product 38 contained small amount of the starting material 36, the mixture of 38 and 36 was subjected repeatedly to glycosylation and then affinity separation to give 38 (α : β = 10:1). ESI-MS m/z 1480.4 [(M+Na)⁺]; ¹H NMR (600 MHz, CDCl₃), α -anomer: δ = 8.07 (2H, NH: barbituric acid), 4.95 (1H, d, J = 3.6 Hz, H-1: Glc_B), 4.53 (1H, d, J = 3.6 Hz, H-1: Glc_A), 4.32 (2H, d, J = 3.3 Hz, H-6: Glc_B), 3.972 (1H, dd, J = 9.3 Hz, 9.3 Hz, H-3: Glc_A), 3.966 (1H, dd, J = 9.1 Hz, 9.1 Hz, H-3: Glc_B), 3.86 (m, 1H, H-5: Glc_B), 3.79 (1H, m, H-6: Glc_A), 3.77 (1H, m, H-5: Glc_A), 3.71 (1H, H-6': Glc_A), 3.64 (1H, dd, J = 9.1 Hz, 9.3 Hz, H-4: Glc_A), 3.50 (1H, H-2: Glc_B), 3.50 (1H, H-4: Glc_B), 3.40 (1H, dd, J = 3.6 Hz, 9.6 Hz, H-2: Glc_A), 3.34 (3H, s, -OCH₃), 3.257 (2H, s, -OCH₂PhCH₂-), 2.48-2.30 (4H, m, -COCH₂CH₂CH₂CO-), 2.19 (2H, CH₃CH₂-), 2.0 (2H, m, -COCH₂CH₂CH₂CO-), 0.92 (3H, CH₃CH₂-). β -anomer: δ = 4.60 (1H, H-1: Glc_A), 4.35 (1H, d, J = 8.4 Hz, H-1: Glc_B), 3.44 (1H, H-2: Glc_A), 3.31 (3H, s, -OCH₃). To a solution of 38 in acetic acid (1 ml) was added Zn-Cu (260 mg) and the mixture was stirred at r.t. for 1 h. Ethyl acetate was added and the insoluble materials were removed by filtration. The filtrate was washed with aqueous saturated NaHCO₃ solution and brine, dried over Na₂SO₄, and concentrated. The residue was then subjected to the affinity separation to give 39. The mixture of 39, phenyl thioglucoside 37 (105.4 mg, 0.147 mmol), PhIO (48 mg, 0.22 mmol), and MS 4A in diethyl ether (4.0 ml) and dioxane (2.0 ml) was stirred at r.t. for 1 h under N₂ and then cooled to -10 °C. TMSOTf (20 μ l, 0.110 mmol) was added and the reaction mixture was stirred for 1 h. The reaction was quenched by addition of aqueous saturated NaHCO₃ solution and ethyl acetate. The organic layer was washed with brine, dried over Na₂SO₄, and concentrated. The residue was dissolved in CH₂Cl₂ and then subjected to the affinity separation to give 119.2 mg of the crude product. The product was purified by silica-gel column chromatography to afford compound 40 (62.0 mg, 28.7%). ESI-MS m/z 1912.6 [(M+Na)⁺]; ¹H NMR (600 MHz, CDCl₃), α -(1-6) α : δ = 8.12 (2H, NH: barbituric acid), 4.98 (1H, d, J = 3.3 Hz, H-1: Glc_C), 4.90 (1H, H-1: Glc_B), 4.54 (1H, H-1: Glc_A), 4.30 (2H, H-6: Glc_C), 3.96 (1H, dd, J = 9.3 Hz, 9.3 Hz, H-3: Glc_C), 3.95 (1H, H-3: Glc_A), 3.94 (1H, H-3: Glc_B), 3.81 (m, 1H, H-5: Glc_C), 3.77 (1H, m, H-6: Glc_A), 3.76 (1H, m, H-6: Glc_B), 3.76 (1H, m, H-5: Glc_B), 3.72 (1H, H-5: Glc_A), 3.70 (1H, H-4: Glc_B), 3.66 (1H, H-4: Glc_A), 3.48 (1H, H-2: Glc_C), 3.48 (1H, H-4: Glc_C), 3.38 (1H, dd, J = 3.0 Hz, 9.3 Hz, H-2: Glc_A), 3.37 (1H, dd, J = 3.0 Hz, 9.3 Hz, H-2: Glc_B), 3.32 (3H, s, -OCH₃), 3.24 (2H, s, -OCH₂PhCH₂-), 2.48-2.30 (4H, m, -COCH₂CH₂CH₂CO-), 2.19 (2H, CH₃CH₂-), 2.01 (2H, m, -COCH₂CH₂CH₂CO-), 0.91 (3H, t, J = 9.1 Hz, CH₃CH₂-).
- (19) The trisaccharide 41 did not exhibit sufficiently strong affinity to the receptor and leaked from the resin column when CH₂Cl₂ was used as an eluent. Large hydrophobic moiety in 41 decreases the relative affinity of the barbituric acid tag with the receptor. When less polar toluene, in which the affinity is expected to increase, was used as an eluent, 41 was effectively retained in the column 1, which was then washed successively with toluene and CH₂Cl₂. Compound 41 was not eluted during the washing. Elution with CH₂Cl₂-methanol (1:1) afforded 41.



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